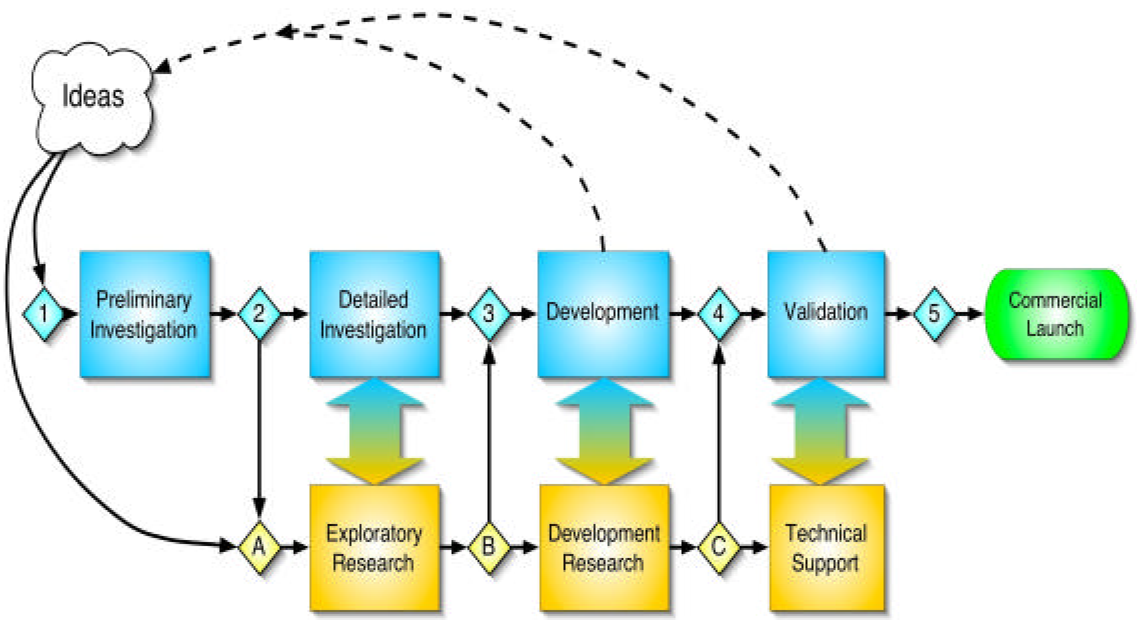
Enzyme Sugar Platform Project
FY03 Review Meeting

May 1-2, 2003

Summary Information



ENZYME SUGAR PLATFORM PROJECT
SUMMARY INFORMATION FOR REVIEWERS

SUMMARY

The Enzyme Sugar Platform (ESP) project seeks to facilitate commercialization of economically attractive cellulose hydrolysis technology for producing sugars from abundant lignocellulosic agricultural residues. Corn stover is the model feedstock. The focus is on core saccharification technology incorporating the next generation, lower-cost cellulases being developed by Genencor and Novozymes. Project success requires breakthroughs in producing cellulases and demonstrating cost-competitive integrated biomass pretreatment and enzymatic cellulose hydrolysis conversion technology. The project began as a commercial-track project within the Stage Gate project management framework being used by the Biomass Program (see cover diagram).

A conceptual corn stover-to-ethanol process and economic model based on continuous dilute acid pretreatment and hybrid hydrolysis and fermentation was used to estimate process costs and assess technical feasibility. Sensitivity studies were performed to identify key parameter sensitivities and important research targets. This information allows us to assess the risk of various technologies. The model assumes that corn stover is available for $35/dry ton, that lower cost cellulases are available (i.e., enzyme development is successful), and that the integrated process performs at target levels believed to be achievable at the pilot scale by 2005. The minimum ethanol selling price (MESP) is estimated to be $1.07/gallon for a plant processing 2200 dry tons of corn stover per day to produce 69 million gallons of ethanol per year. A total project investment (TPI) of $197 million is required, or a TPI per annual gallon above 2.86. This analysis is based on using n^{th} plant modeling techniques and assumes performance levels that are believed achievable in a large-scale plant in 2010. However, the plant could have difficulty competing for financing with dry mill starch ethanol plants that have TPI/annual gallon values near $1.25.

The project's goal was to demonstrate economical process technology for converting biomass to sugars and ethanol. However, at the last review meeting held in Jan. 2002, reviewers recommended focusing on integrating core saccharification technology rather than trying to assemble a full technology package. In another development, the DOE recently awarded Bioenergy grants to six industry/academic/government collaborations to develop technology for the production and utilization of biomass sugars. In light of these developments, the project's mission is changing to one that better supports these industry-led projects. We are thus in the process of transitioning from a commercial to such a research and support track project within the Stage Gate management framework.

This review meeting has several objectives. First, we want to present our recent accomplishments and future work recommendations in the areas of processing modeling and economic analysis, life cycle analysis, corn stover variability analysis, pretreatment, enzyme

hydrolysis and process integration, sample compositional analysis, and partnership development activities. Second, we are soliciting feedback from reviewers and other interested parties on direction and priorities for our work. In particular, does our work add value for the Bioenergy projects and thus align us with the goals of the Biomass Program, and what else can we do to more fully support these and future commercial track projects?

Several recent high-level accomplishments include 1) A first-of-its-kind life cycle model was finalized that describes collection and conversion of corn stover to ethanol in the state of Iowa for use in a light-duty flexible fuel vehicle. 2) Advances were made in techniques and capabilities for rapid compositional analysis of corn stover feedstocks and process intermediates. 3) Many (1000) corn stover samples were collected and over 700 were analyzed and used to assess the effects of genetic and environmental factors on compositional variability. 4) Corn stover compositional variability was shown at the bench and pilot scales to affect hemicellulosic sugar yields and enzymatic conversion of the resulting cellulosic solids. 5) A preliminary kinetic model was developed for enzymatic saccharification of pretreated corn stover.

Our focus will be to expedite success of the industry-led commercialization projects by developing in parallel knowledge and tools that will facilitate their success. Success of the ESP project requires close coordination between many different project participants (e.g., DOE, Genencor, INEEL, Novozymes, NREL, ORNL, USDA, etc.). Beyond the similar need for effective coordination, critical success factors for the success of commercial projects include the existence of appropriate markets, the availability of low cost feedstock, access to cost-effective enzymes and fermentative microorganisms, and the ability to demonstrate robust integrated performance commensurate with compelling economics.

INTRODUCTION

The Enzyme Sugar Platform (ESP) project seeks to advance understanding of enzyme-based cellulose hydrolysis technology focusing on use of a model feedstock (corn stover) and process relevant designs. The Biomass Program conceived the project, initiated in 2000, as a commercial-track project within the stage gate project management framework. At last year's project review, we were given the go ahead to proceed to stage 3, the process development stage of a project targeted for commercialization. The ultimate goal is to facilitate commercialization in the United States with the goal of producing from biomass 10% of our transportation fuel and 18% of our chemicals and materials by 2020 (Roadmap.pdf). However, reviewer feedback as well as the recent Bioenergy awards to cost-share industry-led commercialization projects has changed the focus of this project to a research-track project. The implications of these changes and how we propose to manage the project will be discussed in later sections. This document summaries some of our recent accomplishments and recommendations for future project activities in the areas of life cycle analysis, process modeling and economic analysis, partnership development, sample compositional analysis, corn stover compositional variability assessment, pretreatment, and enzymatic cellulose hydrolysis/process integration. We are soliciting input and feedback from the reviewers and meeting attendees/stakeholders on our proposed activities. Your suggestions will be earnestly considered as we develop our more detailed project plans for fiscal year 2004, which commences in October 2003.

BACKGROUND

Sugar Platform Objective

The objective of the Sugar Platform is to facilitate development of economically compelling process technology for producing fuel ethanol from lignocellulosic biomass based on enzymatic hydrolysis technology by increasing process knowledge and developing tools relevant to the goals of industry. Actual commercialization of the technology will be led by industry, and industrial involvement in the project is critical to its success. The intent is to develop technology for a feedstock resource that is available in sufficient quantities to enable production of over 3 billion gallons of ethanol per annum, i.e., about 1.5 times the total present U.S. production capacity for ethanol produced from grain (principally corn starch). The process technology will exploit the improved cellulase enzymes being developed by Genencor and Novozymes, which are expected to become available for integrated process testing beginning in June 2003 (Genencor) and January 2004 (Novozymes).

Why Enzyme-based Processing?

Competing hydrolysis/fermentation routes for producing ethanol from lignocellulosic biomass include concentrated-acid hydrolysis, 2-stage-dilute-acid hydrolysis, and direct microbial conversion (DMC). Gasification-based conversion followed by fermentation or catalytic conversion are other competing routes in which it is syngas rather than sugars that is ultimately converted to ethanol. Catalytic conversion of fossil fuel-based synthesis gas to liquid fuels (ethanol, gasoline, etc.) is also a competing route. The first two acid hydrolysis-based technologies are the closest to commercialization and are being pursued for niche feedstock opportunities. DMC and synthesis gas conversion technologies have promise in the longer term but are not cost-competitive or sufficiently developed for deployment in the near term. The competitive advantage of enzyme-based technology, as described in greater detail in the Biomass Program's Multi-Year Technical Plan (to be released soon), is that there is greater cost reduction potential for enzymatic cellulose hydrolysis and for enzyme production, as compared to acid hydrolysis-based methods.

Stage Gate Project Management

The Biomass Program is applying "Stage Gate" project management as a tool to improve the quality of its work products. For an overview of the Stage Gate methodology being used, please see the report entitled, "Guide to Implementing the Stage Gate Process for the Biofuels Program" (http://www.afdc.doe.gov/bcfcdoc/5878a.doc). Stage Gate defines two tracks for project development, a commercial track with 5 sequential steps intended for projects that are able to clearly envision commercialization and in later stages are led by industry, and a research track intended for projects where ideas are proven or tested before a commercial project can be visualized (see figure on cover page). Many research projects could become commercial projects when an idea has been fully developed to the point that commercial potential can be envisioned.

In the Stage Gate framework for commercial track projects, process development and commercialization are viewed as a 5-step process. The 5 sequential steps are preliminary and detailed project investigation (Stages 1-2, respectively), process development through pilot scale (Stage 3), demonstration-scale process validation (Stage 4), and finally full-scale production (Stage 5). The intention is for Biomass Program researchers to lead projects in their initial phases including early piloting and scale up (Stages 1-3), with significant industry involvement and guidance in Stage 3, and then to have industry lead the final deployment phases (Stage 4 and 5).

Research projects within the Stage Gate framework are viewed as a 3-step process. The steps are exploratory research (Stage A), development research (Stage B), and technical support (Stage C). Research track projects are coordinated with commercial track projects so that their results can be beneficial to or support these projects.

The ESP project was initiated in the summer of FY00 as a commercial track project. The project passed a Gate 2 review in January 2001, and completed Stage 2 work culminating in a Gate 3 review held January 2002. At the time, there was no industrial partner, however, the project was given the go ahead to start Stage 3 work.

The highlight recommendations of the external panel participating in the Gate 3 review of the ESP project was to focus on developing a better understanding of core saccharification technology and integration issues rather than an integrated process technology package. The reviewers emphasized that once this technology is sufficiently developed, ultimate commercialization will be done by industry, not government. Hence, industry rather than government (national laboratories) should be identifying which specific combinations of feedstock(s), product(s), and processes (i.e., integrated technology packages) appear sufficiently economically compelling to risk the additional costs associated with extended Stage 3 process development and larger-scale Stage 4 demonstration. This feedback has been incorporated into a revised project plan, as described in greater detail in the attached documents. The first file (Comments.pdf) summarizes the reviewers' comments related to each of the Gate 3 review criteria areas, and the second file (Responses.pdf) provides our responses indicating how the project will be revised in response to these comments.

Significant changes have also occurred in the strategy of DOE's Office of the Biomass Program since ESP Project's Gate 3 review was held in January 2002 and DOE's Golden Field Office issued a Letter of Interest in February 2002 to outline its proposed path for moving the ESP Project into Stage 4 and asked industry to explain its preferences for how it would prefer to see this done. In particular, DOE issued a major Bioenergy Solicitation in mid FY02 requesting proposals with 50% or greater cost-share to develop and demonstrate lignocellulose biorefinery-enabling technologies. Awards were announced in response to this solicitation in early FY03. While many details including the schedules remain to be finalized for these DOE- and USDA-funded projects, it is already known that several of them are proposing to develop and demonstrate technologies that incorporate enzymatic cellulose hydrolysis. As a consequence, it is now likely that future efforts to demonstrate and commercialize enzymatic hydrolysis-based conversion technology will occur via these Bioenergy Solicitation projects rather than via the ESP Project as was previously conceived. The full implications of these developments on the ESP Project remain unclear, but clearly the role of the ESP Project is changing.

One of the key objectives of the interim project review is to get feedback from industry stakeholders as to what the role of the ESP project should be in light of these developments. For example, one proposed new role for the ESP Project, which is consistent with the high-level reviewer feedback, is to redirect the project's efforts to focus on fundamental process development and integration rather than on more applied process demonstration, consistent with a research-track project. If redirected in this fashion, the project might be able to broadly support related Bioenergy Solicitation award efforts. For instance, ESP project researchers could develop and apply new tools and techniques that enable improved mechanistic understandings or other as-yet-to-be-identified insights about enzymatic-hydrolysis-based processes to be established. Similarly, fundamental process integration studies could be useful to identify key process interactions that impact process performance and economic feasibility.

ESP Project Scope

Last year's project scope began focused on initiating Stage 3, which seeks to demonstrate integrated process technology that is economically appealing enough for industry to pursue commercialization and continue into Stage 4 and ultimately Stage 5. The project scope was very broad, encompassing everything from feedstock collection, storage, and delivery, to process development and scale-up, to market assessment, to economic analysis and modeling, as well as strategies for commercial deployment. These elements are still important for this project even as a research-track project. It is critical to confirm the technical and economic feasibility of all critical process elements including feedstock availability, feedstock quality, feedstock collection, feedstock pre-conversion processing, feedstock pretreatment, enzymatic cellulose hydrolysis, and mixed biomass sugar cofermentation. Major accomplishments in these areas in the last year are discussed in the next section (Recent Accomplishments).

While DOE/NREL will not commercialize the technology, the key issues must be well understood and addressed early on in all projects to ensure that work remains focused on the most relevant, highest priority tasks (and able to be completed within budget constraints). Rigorous integrated process performance data and supporting information including detailed economic projections must be developed to support claims of commercial viability. This is the best way to ensure that the technology meets a real market need such that the project will have a good chance of successfully moving from Stage 3 to Stage 4. Ideally, based on the compelling performance data and techno-economic projections generated in Stage 3, companies will compete for the opportunity to cost-share and lead the final commercialization steps (Stages 4 and 5).

The heart of this project is process development, i.e., technology evaluation, integration, demonstration, modeling and analysis. A complete enzymatic conversion process encompasses many steps found in numerous existing processes (handling of solid feedstock, ethanol recovery by distillation, steam boiler, various utilities, waste water treatment, etc.) but can be distinguished by its unique core processing steps of feedstock pretreatment, enzymatic cellulose conversion (or saccharification), and biomass sugar fermentation. From a process development perspective, the scope of the project (Stages 2-3) was to select the best combination(s) from among the available pretreatment and enzyme technologies, and then show that selected technology combinations can perform well during extended operation, and hold high potential and manageable risk for demonstrating economic production of fuel ethanol from lignocellulosic biomass. However, this effort must now occur in the commercialization projects. ESP's new role will be to facilitate process development of industry-led projects by focusing on the core technology elements of pretreatment, enzymatic cellulose hydrolysis, and process integration.

Current Status

Corn stover was selected as the model feedstock for initial process development work. Corn stover is estimated to be available in collectable amounts in the U.S. ranging from 60 to 150 million tons annually (dry basis), a far greater amount than for other agricultural residues, and thus best meets the project's original non-niche feedstock criterion. This volume of stover availability is projected to provide sufficient feedstock for production of 4-10 billion gallons per year of ethanol, which is 2-5 times higher than current U.S. ethanol production levels. While other feedstocks ultimately could be selected, corn stover is the most likely feedstock to meet large-scale fuel ethanol demand and is being used in early process development work. Process modeling and economic analysis work currently assumes that corn stover is the feedstock. Note, however, that if another feedstock is subsequently selected, most of the process development knowledge developed for corn stover should readily transfer; differences in the types and levels of trace constituents would still need to be determined for the new feedstock, of course, as would mechanical handling properties and specific processing conditions.

Substantial progress has been achieved in the following areas:

- A new base-line model in ASPEN Plus was completed that allowed key parameter sensitivities to be performed, established integrated performance targets, and provided the basis for Monte Carlo uncertainty analysis.

- A first-of-its-kind life cycle model was finalized that describes collection and conversion of corn stover to ethanol in the state of Iowa for use in a light duty flexible fuel vehicle.

- Advances were made in techniques and capabilities for rapid compositional analysis of corn stover feedstocks and process intermediates.

- Many (1100) corn stover samples were collected and over 700 were analyzed and used to assess the effects of genetic and environmental factors on compositional variability.

- Corn stover compositional variability was shown at the bench and pilot scale to affect xylose yield from dilute acid pretreatment and enzymatic digestibility of the resulting cellulosic solids.

- We improved operation of the 1 ton/d continuous pretreatment reactor to achieve pretreatment conditions of 200°C and operation up to 35% total solids.

- A preliminary kinetic model was developed for enzymatic saccharification of pretreated corn fiber.

- An ethanol marketing study was completed showing that ethanol demand is expected to increase to 4 billion gal/y by 2007 and to 5 billion gal/y by 2012.

This next section of this document highlights the main accomplishments and key findings in each of these areas.

For further background information on the Enzyme Sugar Platform project, please point your browser to:

http://www.ott.doe.gov/biofuels/enzyme_sugar_platform.html
http://www.ott.doe.gov/biofuels/esp_background.html

RECENT ACCOMPLISHMENTS

Summaries of recent work accomplishments are given below. More details are given in the comprehensive write-ups following this summary document.

Process Modeling and Economic Analysis

We studied biomass-to-ethanol process designs and their economics and the results are being used to direct research and to understand potential market penetration issues. This work has also been used as a starting point for corporate partners in their technology development and process design work. Using a process design and parameters that are believed possible in a plant that could be built in 2010 and n^{th} plant modeling techniques, the resulting minimum ethanol selling price (MESP) is $1.09/gal ethanol. Our investigations show that a pioneer, or first of a kind, plant using the same parameter set would have an MESP between $1.53/gal and $1.75/gal (range includes one standard deviation around mean). Sensitivity of MESP to feedstock composition was determined by using 738 different compositions measured as part of the corn stover analysis work (see Corn Stover Analysis section below). The average result was $1.14/gal with a standard deviation of $0.05/gal. Monte Carlo analysis, in which the feedstock composition and important process yields (xylan to xylose in pretreatment, cellulose to glucose during enzymatic hydrolysis, and ethanol yields) are randomly varied, was used to determine a range of possible MESPs. This analysis produced an average MESP of $1.21/gal with a standard deviation of $0.06/gal.

Life Cycle Analysis

We have constructed a life cycle model that describes collecting corn stover in the state of Iowa for the production and use of ethanol in a light duty flexible fuel vehicle. The model incorporates and integrates results from individual models for soil carbon dynamics, soil erosion, agronomics of stover collection and transport, and bioconversion of stover to ethanol. It is the most comprehensive assessment of the environmental, energy and economic impacts of ethanol made from corn stover published to date.

Limitations in available data forced us to focus on a scenario that assumes all farmers in the state of Iowa switch from their current cropping and tilling practices to continuous production of corn and "no till" practices. Under these conditions, which maximize the amount of collectible stover, Iowa alone could produce almost two billion gallons per year of stover-derived fuel ethanol. Soil organic matter, an important indicator of soil health, drops slightly in the early years of stover collection, but remains stable over the 90-year timeframe studied.

We find that a mile fueled by stover-derived ethanol uses 95% less petroleum than a mile fueled by gasoline. Total fossil energy use (coal, oil and natural gas) and greenhouse gas emissions (fossil CO_2, N_2O, and CH_4) are 102% and 113% lower, respectively. Air quality impacts are mixed; with emissions of CO, NO_x and SO_x increasing, while hydrocarbon ozone precursors are reduced. Finally, the model estimates significant economic flows to the rural economy for ethanol prices that are competitive in today's fuel market. More important than the specific results of this study is the demonstrated ability to bridge the gap between advocates of sustainable agriculture and sustainable energy. We see this model as a platform for future discussion and analysis of possible sustainable scenarios for the production of transportation fuels from corn stover and other agricultural residues.

Corn Stover Variability

The chemical composition of corn stover can vary substantially, and this variability can significantly affect processing yields and economics. The primary aim of this work is to characterize the extent of variability in corn stover composition and assess its effect on biomass conversion process economics. A secondary objective is to try to understand the main causes of compositional variation in corn stover, particularly the extent to which variation is caused by genetic and/or environmental factors. The following work was accomplished last year.

- The number and variety of corn stover samples evaluated in FY02 was greatly expanded relative to previous work. Samples from over one hundred genetically distinct commercial corn stover varieties were collected from sites in 10 states (Iowa, Wisconsin, Indiana, Illinois, Minnesota, Ohio, Nebraska, South Dakota, Michigan and Tennessee). Many hybrids were collected from more than one site. In total, over 1100 stover samples from the 2001 crop were obtained in FY02, and the chemical composition of over 700 of them was determined using a robust calibrated near-infrared (NIR) spectroscopic method. Basic statistical methods were employed to characterize the population of samples surveyed.

- Compositional data were evaluated using the process model to estimate the impact of compositional variability in raw stover materials on process economics. The range of carbohydrate content found in corn stover is surprisingly wide and indicates that feedstock composition variability can have a very large impact on process economics. Specifically, the structural carbohydrate content among 738 corn stover samples analyzed varies from 45.3 - 68.5%.

Pretreatment

Pretreatment is a key process technology necessary for liberation of hemicellulosic sugars and for improving the ability of cellulase to enzymatically hydrolyze cellulose, both key requirements for cost-effective conversion of biomass to sugars. An effective pretreatment is also essential to effectively investigate process integration at economically compelling conditions. We perform dilute sulfuric acid pretreatment in a pilot-scale reactor to understand the influence of process-relevant conditions on pretreatment performance. We develop better analysis tools to improve data reliability (e.g., better carbon mass balance closures). We generate pretreated material for process integration work, and to supply subcontractors and stakeholders with material for co-product and residue use studies. Dilute sulfuric acid pretreatment is being used for all of our tasks because we are able to perform this pretreatment at process relevant conditions. We expect the tools and much of the information we develop using sulfuric acid to translate to other pretreatments.

Pretreatment of corn stover was performed in a 1-ton/d continuous pretreatment reactor with different corn stover batches and at a variety of pretreatment conditions. Our original intent was to explore the effect of pretreatment conditions on the hemicellulosic sugar yield and the enzymatic digestibility of the resulting pretreated cellulosic solids. However, the results quickly demonstrated that corn stover compositional variability was significantly affecting performance results. Highlight findings from the pretreatment work are summarized below.

- The project supplied over 5 tons of corn stover, 30 kg of pretreated solids, over 190 L of hydrolysate liquor, and 65 kg of enzymatically-digested process residue to program subcontractors and other stakeholders.

- Experiments with three compositionally different batches of corn stover showed both improved xylose yields and enzymatic conversion of the pretreated cellulose when raw corn stover contained higher levels of total carbohydrates, specifically cellulose and xylan. Confidence testing enabled by repeated runs at the same pretreatment conditions confirmed that these differences were significant. Xylose yields of 80%-85% and enzymatic cellulose conversion of 90%-95% (at a commercial enzyme [CPN] loading of 15 FPU/g cellulose) were achieved with the highest carbohydrate content batch of corn stover.

- We improved the ability of the continuous reactor to operate at high solids concentrations (from 20% to 30%) and obtained performance data at 30% solid concentrations, with a subsequent increase in total sugar (monomeric plus oligomeric glucose, xylose, arabinose, galactose, and mannose) concentration from 94- to 143-g/L. The xylose yields and enzymatic cellulose conversion at 30% solids concentration were comparable to operation at 20% and suggest that in this solids concentration range mass transfer may not be a limitation. However, this is based on very limited data. Significant cost reductions are realized by operating at 30% solids concentration compared to 20% and 30% solids concentration is the target in the process economic models. Within the last two months, we have achieved steady operation at 35% solids, and have initiated experiments to understand the effects of solids concentration on pretreatment performance.

Enzymatic Cellulose Saccharification/Process Integration

The objective of cellulose saccharification/process integration effort is to develop and demonstrate integrated enzyme-based cellulose hydrolysis and to improve the understanding of cellulose hydrolysis via kinetic modeling and experimental investigation. In the large context of process technology, a future goal is to demonstrate the integration of core technologies—pretreatment, saccharification, and fermentation—using corn stover as a model feedstock and to generate high-quality performance data and mass balances for integrated systems operated under realistic conditions. Recent accomplishments are discussed below.

- Enzymatic saccharification of pretreated corn stover solids was conducted at the shake flask and bench scale reactor level. We made progress characterizing enzymatic cellulose saccharification under process-relevant conditions and understanding how to configure the overall process to promote intermediate sugars production. Using pretreated corn stover hydrolysate, the effect of background hydrolysate sugars, primarily xylose and glucose, on saccharification was studied. Different levels of hydrolysate had little effect on glucan conversion using Genencor's Spezyme, whereas with CPN (another enzyme preparation) glucan conversion dropped significantly at high hydrolysate levels indicating susceptibility to increased sugar concentrations. Thus, Spezyme seemed to be less sensitive to background sugar than CPN. Another observation from this study is that final cellobiose levels rise with increasing background sugar, implying a hampering of β-glucosidase performance for both enzyme preparations. Hence, greater resistance to cellobiose inhibition is a desirable trait to aim for in next generation cellulases.

• A kinetic model for cellulose hydrolysis was proposed that differs from previous models in distinguishing between the adsorption of β-glucosidase and that of CBH and EG enzymes. The model also incorporates inhibition by xylose, which has not been considered previously. Xylose is a major sugar in hydrolysates of dilute-acid pretreated biomass. The model performed well in predicting cellulose hydrolysis trends at experimental conditions both inside and outside the design space used for parameters estimation, but it could be improved upon by incorporating the phenomenon of enzyme inactivation and differential hydrolysis potential.

Sample Compositional Analysis

The ability to obtain an accurate chemical composition of biomass and biomass-derived samples using rapid and inexpensive methods is an important enabling technology for the commercialization of biomass conversion processes. Our approach to reducing the time and cost of compositional analysis is to develop rapid analysis methods where multivariate analysis techniques extract chemical information from easily obtained spectroscopic data (near-infrared [NIR]). Such methods provide results in minutes instead of days, a time frame useful for process control at a significantly lower cost ($10-$20/sample instead of $1000-$2000/sample). Work this year led to the improvements in or development of the following methods.

• The accuracy and range of the NIR model for corn stover feedstocks. Specifically the accuracy of minor components (minor sugars, acetyl, ash) was improved and the range of all constituents was expanded.

• The accuracy of the NIR model for dilute acid pretreated dry corn stover solids was improved because of the many additional point added to the calibration database from pretreated samples generated by ESP and Advanced Pretreatment project work.

• A preliminary NIR model for pretreated corn stover solids in the presence of hydrolysate liquor was developed that eliminates many of the sample preparation steps associated with the dry solid measurement and is an initial step toward a model that can be used for on-line measurements.

• A preliminary NIR model was also developed for sugars and other soluble components in hydrolysate liquor derived from pretreated corn stover and again is an initial step towards on-line measurements.

Partnership Development

The partnership development program of the sugar platform becomes more vital as the processes move closer to commercial viability. Through recent colloquies and meetings, industrial partners have provided valuable insight to DOE and the Biomass Program on how to structure planned cost-shared technology pilot scale demonstrations, leading to the highly successful DOE 2002 Biomass R&D solicitation that resulted in six awards with nearly $80 million in industrial cost-share.

The partnership development team also undertook a major market study to enumerate and analyze the political and environmental conditions that are changing U.S. ethanol markets, the range of projected demand levels 2003 – 2012, and predictions of price ranges due to changing markets. This study found that U.S. production and consumption of ethanol is expected to rise sharply in the period 2004 – 2012, reaching nearly 4 billion gallons/year by 2007 and 5 billion gallons by 2012. This sharp increase in demand is driven primarily by the planned phaseout of MTBE in major gasoline markets and secondarily by the possible passage by Congress of the Renewable Fuels Standard (RFS). This sharp increase in demand will provide upward pressure on ethanol prices, with consensus estimates being that ethanol prices will reach $1.50/gallon by 2006 – 2010 (depending on assumptions on market demand, as well as state and local mandates). Rising ethanol production levels will in turn put upward pressure on corn prices, with even the most optimistic estimates see corn prices rising from $2.10 – 2.20/bushel today to $2.60 – $3.00 within ten years.

The proposed phase out of MTBE by itself will put sharp upward pressure on grain-based ethanol prices in the period 2003 – 2006 while new ethanol production capacity is being built. The higher predicted market prices for ethanol and corn will make the business case for cellulosic ethanol much more attractive throughout the whole period 2004 –2012. High ethanol prices, rising corn prices and surging DDG production may also encourage grain ethanol producers to experiment in the near-term with the addition of significant cellulosic biomass conversion trains at existing or new dry mill facilities, using DDG as the feedstock**.**

Based on industry request, NREL is considering the development of a new industrially-led consortium that we are tentatively calling the Biomass Rapid Analysis Network or BRAN. While the Board of Directors will determine the activities of the BRAN, services that have been indicated as high priority by potential industrial participants include:

- 2 and 5 day training programs on biomass analysis
- Development of standard data collection and analysis protocols
- Creation of new multivariate analysis equations based on NIR
- Sample preparation
- Data clearinghouse for BRAN members
- Software testing
- Equipment testing

How this proposed organization would be structured will be subject of detailed discussions with interested industrial and research partners.

In support of the Sugar Platform, NREL worked with two sub-contractors to look at the feasibility and economic viability of co-locating a cellulosic ethanol plant with a current or planned coal-fired power plant. The studies looked at two power plants in the Mid-West and one in New England to determine such key variables as the feedstock availability and cost, the impacts of the co-location on key ethanol plant capital and operating costs, proforma and economic analysis of required investments, any substantial environmental and social impacts, and markets for the plant's ethanol production. At all three sites, significant amounts of corn stover were available for ethanol production, at assumed delivered costs from $33 – 38/bone dry ton. The available corn stover at the three sites would support an ethanol plant ranging from 20 million gallons per year in upstate New York to more than 70 million gallons/year at a proposed power plant site in Nebraska.

The site-specific studies found that the internal rate of return (IRR) for two of the sites were quite reasonable – 18 – 23%/year or better – for larger size plants (50 – 70 million gallons/year) or when the proposed ethanol plant could make use of some of the coal-fired power plant's existing infrastructure. Sensitivity analyses revealed that the IRRs for the sites studied were quite sensitive to a number of factors, including: the price of the corn stover feedstock, the ethanol yield (in gallons/bone dry ton of feedstock), the sales price of the ethanol, and the percentage of equity investment in the plant. In general, delivered corn stover feedstock costs in the $25.00 – 30.00/ton range greatly increased the profitability of the proposed ethanol plant, as did ethanol selling prices above $1.25/gallon. The IRR of proposed co-located ethanol plants also increased sharply when the proposed equity investment dropped below 60% of the total project costs.

RECOMMENTATIONS

Stage Gate Management

As previously discussed, the project role is changing from demonstration of process technology typical of stage 3 commercial track projects to a focus on saccharification technology and integration issues. Our goal is to support recent and future Bioenergy awards by developing tools and increasing our mechanistic understanding of an enzyme-based biomass conversion process to decrease the cost and risk of this technology. Therefore, we are realigning the project structure and emphasis to be consistent with a research track project in Stage B. A description of the goals, activities, and output of a Stage B project are given in the Appendix A, and review criteria are in Appendix B. Consistent with this approach, further work in the areas of life cycle analysis, process engineering, and partnership development would not be directly conducted within the ESP project, although core Biomass Program work in these areas would continue.

This review is not a formal gate review, but we will discuss and evaluate progress according to Gate B review criteria in light of changes to a research track project. This discussion is presented after the recommendation section.

Recommendations for ESP Project

Recommendations for the ESP project are listed below and encompass both near- and long-term recommendations. Although all of the goals are important, we feel that the near-term recommendations provide the best immediate value to the Biomass Program.

Corn Stover Variability

<u>Near-term</u>

1. We do not yet understand the major causes of compositional variability in corn stover. While we have some evidence that both genetic and environmental factors play a role, it would be worthwhile to understand enough about this system to be able to manage compositional variability (if only partially). The ability to manipulate stover composition to some extent through changes in agronomic practice or breeding could provide higher quality feedstocks for biomass conversion processes. A joint effort between DOE, USDA and selected agronomy departments at major universities is recommended to study this issue, to identify the major causes of stover variability, and to develop strategies to enhance stover quality for biomass conversion. This effort would leverage resources in ongoing efforts in the field of forage and silage quality that the USDA already funds at universities and its own laboratories.

2. Several hundred more stover samples are already on hand at NREL that are presumed to represent a greater range of genetic diversity than is present among the commercial hybrids surveyed in the initial study. From the perspective of trying to understand the breeding potential of corn in terms of stover composition, it would be worthwhile to see if a more exotic segment of the corn germplasm extends the range of compositional variability seen in this work. It is recommended that these stover samples be processed and analyzed for inclusion in the corn stover composition database. Depending on the results of this work, it may be worthwhile to explore corn germplasm collections in greater depth over a period of a few years. This would be done in conjunction with the USDA's National Plant Germplasm System.

3. Begin work to establish and maintain strong relationships with corn breeders and agronomists at public and private institutions. These organizations are in a position to implement genetic strategies and alter cultivation and harvesting practices that can enhance the overall quality of corn stover (without sacrificing grain yield). The objective is to increase the awareness of seed companies, breeders and agronomists that biomass conversion is a business opportunity that they can contribute to developing.

<u>Long-term</u>

4. The variety of corn hybrids offered to farmers each year is constantly changing as new hybrids are released and older varieties are withdrawn from the market. The average market lifetime of a hybrid is probably on the order of 5 years or so, and we need to

understand if and how stover composition is changing over time. It is recommended that this work be carried out in conjunction with state university-run grain yield trials.

5. Different stover materials sometimes exhibit different processing characteristics. This has mostly manifested itself as differences in pretreatment and enzymatic hydrolysis yields, but we currently do not understand what causes differential processing efficiency. It could be due to structural differences in cell walls between hybrids, suggesting that cell walls may be architecturally distinct. We currently have very few tools available that can help to distinguish such differences. It is recommended that the Biomass Program devote resources to exploring the use of tools that can distinguish differences in cell wall architecture (e.g., via antibodies, cell wall degrading enzymes, microscopic techniques, NMR and other techniques). In addition, differences in processing efficiencies could be artifacts of the way in which stover is harvested, handled, stored or processed prior to introduction into the processing facility. In this case it would be useful to document the complete history of every bale of stover that comes in so that we can attempt to correlate processing behavior with sample history. As these two alternatives are not necessarily mutually exclusive, it is also recommended that the Program establish and maintain a database of feedstock materials that records all pertinent information about those materials, from planting through harvest and storage.

Pretreatment

<u>Near-term</u>

6. Continue to generate and supply raw, pretreated, and enzymatically-digested materials to program subcontractors and other external stakeholders for co-product and residue use studies.

7. Advance knowledge about high-solids, dilute sulfuric-acid pretreatment (25%-30% solids) using corn stover as the model feedstock. Specifically work is planned to 1) determine the impact of high solids pretreatment on hemicellulosic sugar yields and enzymatic cellulose conversion; 2) coordinate with the Advanced Pretreatment project to improve overall pretreatment mass balance by applying newly developed compositional analysis tools for quantifying soluble protein and uronic acids; and 3) explore the relationship between enzymatic cellulose conversion and the physiochemical properties of pretreated biomass. We propose to investigate and develop new tools and methods to understand how the fine structure of pretreated biomass affects enzymatic hydrolysis.

<u>Long-term</u>

8. Extend investigating the effect of corn stover compositional variability, particularly the carbohydrate levels on pretreatment performance. We recently obtained batches of several different corn stover varieties exhibiting a range of compositions arguably more representative of the range of stover compositions expected at the plant gate; our previous material had been pre-processed (washed and milled) and to this extent may not have

been fully representative. We propose to expand our investigation of the effect of compositional variability using these more representative materials.

9. Continue close coordination with the Advance Pretreatment task to implement new concepts for improving pretreatment performance in the 1 ton/d pilot scale reactor, as and when they are proved to be cost effective and ready for scale up.

Enzymatic Hydrolysis/Process Integration

Near-term

10. As Genencor International and Novozymes Biotech Inc. develop improved enzymes these need to be evaluated for their ability to hydrolyze cellulose at process-relevant conditions and assessed for their ability to meet aggressive conversion/rate goals consistent with process engineering model targets. The projected costs from the economic model are based on certain assumptions. It is imperative to assess how the improved enzymes perform in relation to these criteria and to identify ways to most efficiently use these enzymes. This work will build upon process knowledge gained from integrated processing research discussed below.

11. Process integration requires effective hydrolysate conditioning methods to enable good saccharification performance to be achieved. Conditioning studies are needed to better understand carbon and other component behaviors (e.g., fate of Ca and S) and to increase the accuracy and rigor of our process simulations.

Long-term

12. Engineering data for processes involving solid-liquid separations of hydrolysate and fermentation residues are needed to improve process engineering models. We propose to use our capabilities for generating large quantities of these materials to develop the necessary data. This work will be coordinated with the process engineering team and their relevant subcontracts, and will make use of new separation equipment (Pnuempress filter) assumed in our recent process designs.

13. The enzymatic saccharification strategy (SSF [simultaneous saccharification and fermentation], SHF [separate hydrolysis and fermentation], or HHF [hybrid hydrolysis and fermentation]) is highly dependent upon the enzyme/microorganism system. We propose to integrate saccharification and fermentation using model microorganisms (e.g, glucose fermenting yeast and/or recombinant bacteria) to integrated saccharification and fermentation to study the factors influencing cellulose hydrolysis. This work will help us to further develop the tools and methodologies to improve carbon and mass balance closure across the saccharification/fermentation unit operation.

14. Working at relatively high solids levels poses mixing challenges. Studying the rheological properties of corn stover slurries and exploring the impact of mixing on saccharification performance at high solids levels will advance general knowledge about

the overall process. Related to this is the effect of increasing shear forces caused by the need for mixing high solid slurries on cellulase deactivation.

15. Render the kinetic model for enzymatic hydrolysis more useful by incorporating enzyme inactivation and hydrolysis capacity factor. The feedstock hydrolysis capacity factor will be used to account for variable digestibilities in different lots of pretreated corn stover. Enzyme inactivation also needs to be incorporated to account for gradual enzyme inactivation during prolonged exposure to relatively high temperatures. A more robust model incorporating these features will be useful for *in silico* process optimization.

Recommendations for Sample Compositional Analysis

In support of the anticipated needs of an emerging biomass conversion industry we propose continued work in the following areas:

<u>Near-term</u>

16. Improve wet chemical methods for biomass feedstocks (current emphasis is on corn stover) and biomass-derived materials need to be developed, validated and published as standard methods. Since rapid analysis methods retain the precision and accuracy of the wet chemical methods upon which they are calibrated, improving the calibration methods is necessary for improved rapid methods. New wet chemical methods (emphasis on protein and uronic acids) need to be validated and QA/QC protocols established before the methods can be published as standard consensus methods. Publication of the validated standard methods provides the industry with a common ground for comparison of various feedstocks and conversion processes. We propose to advance the state of wet chemical methods through both core and subcontracted research.

17. New analytical methods in one or more of the following areas, protein analysis for pretreated biomass, characterization of uronic acid and pectin in biomass, cutin characterization, and studies on the mechanism of overliming, will be developed under subcontract to academic institutions.

<u>Long-term</u>

18. Continue to broaden the range of rapid analysis methods using improved wet chemical methods in conjunction with a variety of spectroscopic techniques, spectrometers and multivariate analysis software packages. Procedures are being developed to standardize spectra collected on different instruments and to accurately transfer PLS equations between instruments and software packages. Validation of core rapid analysis methods can be done at the point of method development or across an industrial group. Deployment to industrial partners will require customizing and validating these methods in the new setting. Understanding the challenges associated with calibration transfer is key to successful technology transfer.

19. Rapid analysis methods are already being used at NREL to support ongoing research on dilute acid pretreatment of corn stover. These corn stover rapid analysis methods are currently laboratory based, but planning has begun to develop at-line and on-line versions for demonstration in the DOE/NREL pilot plant. Deployment and testing of rapid biomass analysis methods at the pilot scale should provide valuable information about the challenges associated with on-line measurement. At the same time, information will be obtained about the use of these methods to monitor and control biomass conversion processes in real-time.

Recommendations for Other Sugar Platform Work

Process Modeling and Economics

Areas for near-term investigations to reduce uncertainties in the process modeling simulation and economic projections include:

<u>Near-term</u>

20. Improve understanding of all gaseous, wastewater and solids emissions generated by a bioconversion facility and investigate alternative process designs and permitting requirements that better manage or preferably eliminate emissions.

21. Investigate improved biomass handling and storage designs better matched to probable agricultural practices for corn stover collection and storage.

22. Improve model material balance capabilities by adding various components (e.g., protein and uronic acid) not currently in the model (requires parallel analytical methods development) as well as matching compositional information with elemental composition.

23. Add experimentally determined solid/liquid separations parameters to the process model to improve its predictive capability.

24. Improve understanding of conditioning processes, in particular overliming, by incorporating experimental data as well as more rigorous modeling of reactions, solubilities, and electrolyte properties.

<u>Long-term</u>

25. Optimize biorefinery operation using LP (linear programming like) models.

26. Add kinetic models into the existing process simulation models to improve sensitivity analysis and prediction of yields.

27. Extend work on risk analysis by assessing errors in the economic modeling methodology and adding the latest information on experimental errors in parameter estimates.

28. Continue to track technology progress by incorporating the latest data and understanding gained from experimental work.

Life Cycle Analysis

The LCA work represents a first step in learning how to look at soil sustainability issues related to collecting agricultural residues in a life cycle context. To expand this work to a level of practical use for policy makers and for industry, all the following recommendations are needed:

29. Redo the Iowa analysis to include a long-term projection for soil carbon levels and greenhouse gas emissions for the current mix of tilling and cropping practices.

30. Evaluate stover collection impacts for the dominant crop rotation of corn and soybean production in Iowa.

31. Expand our analysis to other states, especially to Indiana and Nebraska for which soil carbon inventories have now been completed by CSU-NREL. Ultimately, we should expand the analysis to cover the top ten corn and wheat producing states. The objective of this expanded analysis is to provide a better picture of the potential impact agricultural residues can have on U.S. energy supplies and greenhouse gas emissions.

32. The analysis should be expanded to include wheat straw.

33. We should analyze scenarios in which switchgrass and other native grasses are introduced into the rotation cycle.

34. Water quality impacts need to be considered. The problems of water impacts from agriculture are very high on the list of concerns raised by environmental organizations. The current modeling should be expanded to include leaching of nutrients from the soil into groundwater and pollution of surface waters due to water runoff.

Partnership Development

Areas of continued activities for partnership development for the Sugar Platform include:

35. Finding industrial partners to assist in the needed cost reductions and technology scale-ups in a number of key ways: locating and securing low-cost feedstocks (some of them captive residue streams within existing processing plants) for the initial technology demonstration plants, finding co-location opportunities that will reduce the required capital costs for demonstration plants, identifying high value co-products from the sugar streams that will increase the economic viability of the planned biorefinery, and finding new markets for existing Sugar Platform products and co-products.

36. The major challenge in the use of rapid biomass analysis is the cost of method development, estimated to be around $300,000 per method. Since these methods are

feedstock and process specific, several methods may need to be developed to adequately monitor a process and core calibration may need to be customized for different process environments. One proposed solution to this challenge to organize an industry-wide rapid analysis network that collectively shares the cost of method development. Core methods could be developed, validated and published as standard consensus methods. This network could facilitate communication for troubleshooting and problem solving and provide training opportunities in wet chemical calibration methods, advanced spectroscopic techniques, multivariate analysis, QA/QC procedures and long-term method maintenance. Participating members could look to the network for the latest information on instruments, multivariate analysis software, calibration transfer, and calibration customization.

37. Understanding the high-level economics of prospective coal co-locations may help reduce the capital/commercialization hurdle for a pioneer plant. However, a more detailed technical feasibility study is required hopefully with in-kind funding from the power company. We are interested in reviewer's comments concerning the usefulness of continuing this work.

REVIEWING STAGE GATE CRITERIA

The objective of this review is to ensure that the project remains aligned with Biomass Program objectives and is on track to meet its Stage Gate goals. Therefore, alignment of project elements to the appropriate Stage B criteria is addressed below. The reviewers are encouraged to assess our recommendations or to propose new work elements for the ESP project within the context of these criteria.

Strategic Fit

Questions: Does the proposed research build knowledge or capability in alignment with program goals?

This project advances our understanding of the core technologies to enable lignocellulose-based biorefineries that produce a slate of commodity and specialty fuels, chemicals, materials, and energy (heat, electricity) products. This project explores and developed tools to enable more efficient development of sugar platform enzymatic cellulose hydrolysis-based conversion technology. The core pretreatment and enzymatic saccharification technology will also enable other bio-based products and co-products to be produced, most notably co-products that would be derived from lignin or biomass sugar components that can be recovered from various processing streams. (Identifying and developing specific co-products and co-product markets will spur technology deployment but is viewed by USDOE's Biomass Program as an area that industry must lead.) The enzymatic saccharification technology utilizes the improved cellulase enzyme technology (substantially lower cost cellulases) currently being developed under DOE subcontract by Genencor and Novozymes, the world's leading producers of industrial enzymes. Developing and deploying improved biomass sample compositional methods also will be valuable to industry and thus meets the Biomass Program's strategic goals.

Customer

Questions: Who are the customers for this knowledge, and is this information valuable to the customers?

ESP project work and work in biomass-derived sample compositional analysis directly supports enzymatic hydrolysis-based commercial track projects, including several of the Bioenergy awards. Our research focus is to develop better methods and capabilities that through their application will enable us to gain a deeper level of understanding of biomass conversion. ESP project work focuses on the critical success factors related to feedstock, high-solids pretreatment, and high-yield sugar production. Advances in each of these areas will reduce the risk to industry in demonstrating and deploying sugar platform technologies.

Sample compositional analysis work is focused on developing rapid analysis tools that will enable feedstock valuation as well as conversion process monitoring and control. Such methods will be needed to establish first-generation conversion plants. This work as proposed in recommendations 16-19 is intended primarily to meet the needs of the developing industry.

Another demonstration of this project's customer focus is that we have supplied large quantities of raw, pretreated, and enzymatically-digested materials to many industry stakeholders. This work is important and we propose to continue it (Recommendation 6).

Technical Feasibility and Risk

Questions: Is the research approach feasible and what are the technical risks in investing in this line of research or developing this capability?

Technoeconomic analysis of an enzyme-based lignocellulosic conversion process indicates that it is theoretically possible to produce ethanol (or other products) at reasonable costs, i.e., at cost that are competitive with existing starch-based ethanol. (See discussion on process modeling and economics in Recent Accomplishments). Work will continue in process modeling and economic analysis and in placing engineering subcontracts. The objective will be to continue to increase the technical feasibility and to reduce performance risk. This work is proposed in recommendations 20-28.

The ESP project will continue to investigate the major unit operations under process realistic conditions to increase the knowledge base and identify key issues. We will also continue to develop tools. Together these efforts will help to significantly reduce economic and performance risks. Much of this work is addressed by the critical success factors discussed below. However, there is some risk in this work leading to economically viable technology. Beyond this, at least two process cost centers – feedstocks and cellulase enzymes – are being addressed outside of the core project. However, ORNL, INEEL, and USDA are developing approaches that enable lower delivered feedstock costs, which helps reduce the risk associated with feedstock cost. Substantial progress is being achieved by Genencor and Novozymes to reduce the risk of lower cost enzymes being available. Implementation of new pretreatment concepts or alternative pretreatment that are effective, reduce cost and pose less risk would improve technical feasibility (Recommendation 10), but appropriate Gate criteria must be met first. There is little risk in continuing sample compositional analysis as this information and tools developed will be valuable for any technology conceived for biomass conversion (Recommendations 16-19).

Competitive Advantage

Questions: Will this knowledge or capability improve the likelihood of commercial success and what other research or development routes exist and what are their relative chances of success?

Please refer to the "Why Enzyme-based Processing?" in the background section and "Strategic Fit" above for a brief description of alternative routes to producing ethanol from biomass feedstocks and fossil fuel resources; why we believe enzyme-based processing has the most long term cost reduction potential for very large volume production; and why the ability to enable multi-product biorefineries distinguishes this technology from most of the competition. Extensive process modeling and economic analysis show the promise of enzyme-based processing.

Legal/Regulatory Compliance

Questions: Are there patent, emissions, safety, and permitting issues to consider and are they surmountable?

Many details of the process remain incompletely defined, so it is premature to be able to make complete recommendations in this area. The issues as discussed below are significant but not insurmountable. Our objective in Stage B is to identify and explore at a preliminary level the issues that will need to be addressed more concretely in a more clearly defined Stage 3 commercial track project. Although these issues will not be addressed by the ESP project, there are important elements that can be researched as part of other sugar platform projects. There are at least three areas – feedstock collection, bioconversion, and waste disposal – where important legal or regulatory issues need to be considered:

1. The environmental impact of biomass feedstock (currently corn stover) collection on soil erosion, soil carbon levels, and overall soil sustainability needs to be understood to support policies promoting feedstock removal. The USDA soil studies and multi-institutional lifecycle work are intended to address this issue. Preliminary results suggest that some corn stover can be collected without detrimentally impacting soil health, but much more remains to be done to understand this issue more fully, i.e., in relation to soil type and cultivation practices (See recommendations 29-34 for continued life cycle studies).

2. Issues related to licensing patented pretreatment, enzyme production, fermentative microorganism, or other technologies, as well as obtaining permits for very large scale use of genetically modified microorganisms, especially in the ethanol fermentors, needs to be carefully considered and resolved. This is particularly true in the case where biomass sugar production (i.e., enzymatic cellulose hydrolysis) and biomass sugar fermentation are decoupled (as, for example, in a process based on sequential hydrolysis and fermentation

[SHF]), where it is anticipated that the spent cell mass could be recovered to provide value-added coproducts. Although, we will not pursue any work in this area in this project, these issues are critical to the success of any commercial track project.

3. Potential byproduct or emission issues related to productive use or disposal, respectively, of gypsum waste, and potentially for other potential process waste streams as well, need to be fully considered. There is potential that the phenolics content (derived from solubilized and precipitated lignin) of gypsum waste may require this material to be regulated as a hazardous waste. Further work needs to be done to integrate the process and produce representative gypsum waste and other process wastes for analysis. We proposed to continue work in the area using engineering subcontractors (Recommendation 20) as well as in-house research on conditioning (Recommendation 11).

Critical Success Factors and Showstoppers

Questions: Have critical success factors and showstoppers been addressed and what are the plans to address them?

The Biomass Research and Development Technical Advisory Committee has produced a roadmap document (Roadmap.pdf) outlining the technical barriers to the development of biorefinery-based production technologies. Consistent with this message, DOE's Office of the Biomass Program draft Multiyear Program Plan 2003 to 2010 has elaborated on these technical barriers or knowledge gaps that are particularly relevant to the mission of the ESP project. We have further refined the list and some of these key performance and cost barriers are listed below. Although this project cannot address all of these factors, we have noted how proposed areas of investigation relate to or address some of them.

Feedstock

Feedstock Valuation and Delivery: *Lack of information on the feedstock supply infrastructure encompassing lignocellulose valuation, collection, storage, and delivery. Knowledge gaps exist on how best to do this.*

Recently completed work documents the variability of corn stover, as well as its implication on process economics. Recommendations 1-5 are intended to increase our knowledge in areas such as sources of feedstock variability, developing genetic improvements, and better understanding the impacts of cultivation and harvesting practices. This type of work requires significant outside participation by USDA and others to be successful (Recommendations 2-4). This work will also be done conjunction with process engineering studies (Recommendation 21).

Sugar (and Lignin) Production

Analytical Methods and Sensors: *Lack of validated methods and sensors for determining the composition and structure of biomass and process intermediates. (This issue implicitly embedded in production barriers listed below.)*

Significant progress has been made in the development of rapid biomass analysis methods, as well as on validation and distribution of current wet chemical methods for biomass analysis. Further, method development, validation, and distribution will be done as proposed in recommendations 16-19.

Pretreatment of Biomass: *Information is needed on the root causes of biomass recalcitrance; the role of biomass structure and composition on pretreatment performance; pretreatment process chemistries and reaction kinetics and equipment reliability and materials of construction requirements (MOC) for the most promising technologies; all under realistic high-solids operating conditions.*

Results to date show that corn stover compositional variability has a significant effect on hemicellulosic sugar yields and enzymatic cellulose conversion, but effects of high-solids pretreatments are still unknown. To address this issue, we have improved the ability of a 1 ton/d pretreatment reactor to be operated at industrially-relevant high-solids conditions. Recommendations 7 and 8 are aimed at improving core pretreatment knowledge in the area of high-solids pretreatment and extending knowledge on the effect of corn stover compositional variability on performance.

Enzymatic Hydrolysis: *Enzymatic hydrolysis is being limited by the low specific activity of current commercial enzymes; high cost of enzyme production; lack of knowledge of how biomass structure and composition influence reactivity; and an insufficient understanding of fundamental enzyme biochemistry and cellulose hydrolysis mechanisms.*

A preliminary kinetic model for cellulose hydrolysis has been developed that builds upon previous models by incorporating xylose inhibition and being able to distinguish between adsorption of ß-glucosidase and CBH/EG enzymes. We propose to incorporate enzyme inactivation and a hydrolysis capacity factor (i.e., susceptibility of the cellulose to enzymatic hydrolysis) into the model (Recommendation 15). In addition, the pretreatment work discussed above will provide material that can be used to develop a better fundamental understanding of how pretreated biomass structure affects enzymatic cellulose hydrolysis.

Process Integration: *Process integration ties together elements of the individual barriers identified above but is further complicated in that it includes linking unit operations which often involves additional separation processes (e.g., solid-liquid separation). There is a lack of understanding of key process interactions between feedstock, pretreatment, enzymatic hydrolysis, and fermentation at process relevant conditions. Experimental systems are needed to understand these interactions and to produce high quality performance data. Further understanding could lead to the development of kinetic models for incorporation into dynamic process models.*

Fundamental knowledge on process integration is lacking. We have begun to explore the relationship between feedstock, pretreatment performance, and enzymatic hydrolysis, but are clearly at the beginning of this effort. In addition to the pretreatment work discussed above that also supports process integration efforts, we propose work on conditioning, biomass conversion related separations, influence of process configuration on cellulose hydrolysis, and reactor design studies (Recommendations 11-14).

Utilization (a. k. a. Bioproducts)

No work has been proposed in the utilization area for this project. Much of this effort would occur in a commercial track project.

Bioproduct Strategy: *There is a lack of clear targets and prioritization criteria for this area and is clearly an area that industry must lead. (This issue implicitly embedded in utilization barriers.)*

Fermentation Microorganisms: *Microbes are needed that can utilize all biomass sugars; exhibit robustness to demanding industrial conditions (low pH, high temperature, toxic or inhibitory compounds, etc.); and are acceptable in food related process settings. Additionally there is a lack of "-omics" data that is hindings current development efforts.*

Bio/catalysis: *There is a lack of catalyst systems that provide high selectivity to the desired product; and a lack of a detailed mechanistic understanding of aqueous phase catalytic reactions.*

Lignin Utilization: *A lack of knowledge about process lignin's value for direct combustion and/or gasification, or as an ingredient in higher value coproducts.*

Appendix A: Stage B Description

Goals:

- Answer key technical questions in order to gain an understanding how to best tackle the major scientific challenges in developing program technologies.
- Develop technical, scientific and/or engineering capability critical to the success of the commercializing technologies under development by the Program.

Stage B Work Activities:

Stage B will build upon the exploratory knowledge or capability gained in Stage A in a focused, detailed experimental program. We are investing in developing the scientific knowledge and capability that will enable us to answer important scientific and technical questions in the future. While the work is not directly related to commercialization objectives, ultimate <u>success will be measured by the degree to which this new knowledge or capability is used in new or existing commercially focused projects.</u>

- **Market Assessment**
  - Reconfirm importance of scientific questions to be answered or capabilities to be developed, and focus the efforts to solve the specific problems or issues identified through discussions with other project teams and/or potential industrial partners.

- **Research Activities**
  - **Validation of selected research strategy**
    - Validate that the research strategy has an excellent chance of success when applied to the specific problem to be solved.
  - **Carry out planned work to solve specific problem(s) identified**
    - Since it is the intention that the results of this research benefit a defined (Blue Line) project, involvement with a partner involved in a defined project is appropriate.
    - Monitor progress in knowledge milestones that yield key pieces of the information necessary to meet the ultimate goal. In many cases, it should be possible to attach a specific performance objective to a knowledge milestone
  - **Detailed Technical Assessment**
    - Capture value of gained knowledge or capability developed in milestone reports.
    - Continue to compare progress to other known activities in the area.

Stage B Outputs:

- **Market Assessment**
  - Updated customer assessment of relevance and importance of working on the specific scientific question(s), or developing the new capabilities.
- **Research Results**
  - Detailed documentation of all relevant experimental work.
  - Publication and dissemination of information gained to the widest possible audience.
- **Detailed Technical Assessment**
  - Summary of how the understanding or capability can be applied to commercially focused projects, including updated knowledge gaps with plan of action if appropriate.
  - Review of the new information to determine impact on other program research activities to identify possible changes to ongoing or planned activities and/or shifts in program emphasis.

- Identification of "lessons learned" from the project. What went well and what did not? etc.
- Recommended next steps. This would be covered in a Gate C review in which the primary focus is the plan to transfer the newly developed technology or scientific capability to those projects or partners who can use it.

Appendix B: Stage B Review Criteria

- **Strategic Fit**
- Does proposed research build knowledge or capability in alignment with program strategic direction?

- **Customer**
- Who are the customers for the new knowledge or capability?
- How will the knowledge or capability developed be valuable to the customer, or essential to future commercialization?
- What is the customer's perception regarding the relevant "window of opportunity" for this work?
- What are business risks to investing in this line of research or developing this capability?

- **Technical Feasibility and Risks**
- Is research approach feasible for the specific problem identified?
  - Describe how proposed work is a departure and is an improvement from current research pathways, including work outside of NREL.
  - What are the technical risks in investing in this line of research or developing this capability?

- **Competitive Advantage**
- How will proposed knowledge or capability improve the chances of commercial success?
- What other research or development routes exist and what are their relative advantages and disadvantages?
- What could happen to make this area of development research, or capability obsolete?

- **Legal/Regulatory Compliance**
- Patent positions
- Waste steams, emissions, safety, permitting
- Are issues surmountable?

- **Critical Success Factors and Showstoppers**
- Provide prepared list of success factors and showstoppers.
- Have critical success factors been addressed?
- Plans to address potential business and technical showstoppers.

  - **Plan to Proceed**
- What are detailed plans for Stage B (with knowledge milestones, schedule, and resource estimates)?
  - What are general plans for use of the knowledge gained or capability developed by customers or commercially focused projects?

Gate B Keepers
- NREL Technical Manager
- DOE Program Manager
- NREL Technical Lead

Customers, Industry Partner(s)